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Submission date: 24-Jun-2023 10:56AM (UTC+0700)

Submission ID: 2121719374

File name: Domestication of Blue Swimming Crab for Sustainability_ Growth and Genetic Changes.pdf
(459.05K)

Word count: 4104

Character count: 21748

Domestication of Blue Swimming Crab for Sustainability: Growth and Genetic Change

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Abstract

Blue swimming crabs (*Portunus pelagicus*) are a fisheries resource which is currently threatened by over-exploitation. Crab culture is one approach which can contribute towards sustainable use of blue swimming crab (BSC) populations. In this context, BSC domestication is considered necessary. The purpose of this study was to evaluate the genetic and growth changes occurring during initial stages of a BSC domestication program. The DNA of selected strains was examined using Random Amplified Polymorphism DNA (RAPD). Five OPA primers were used to generate a fragment pattern from the samples collected. The RAPD results showed that BSC domestication combined with selection for fast growth resulted in genetic change, with a genetic distance of 0.1053 – 0.3158 between 1st and 4th generations. This distance was associated with significantly higher growth rates after the 4th generation. After 100 days grow-out, mean growth in carapace width was 103 mm for 1st generation crabs and 109 mm in the 4th generation, with respective growth rates of 1.0 mm/day and 1.1 mm/day. Domestication with selection of fast growing individuals as BSC broodstock resulted in a positive shift towards DNA characteristics associated with faster growth in subsequent BSC generations.

Keywords: Blue swimming crab, Sustainability, Domestication, Selective breeding, Growth rate, RAPD

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1. Introduction

The blue swimming crab (*Portunus pelagicus*) is a globally important fisheries resource. In Indonesia, this crab is found in most coastal waters within the archipelago, but in recent years indications of over-exploitation have been reported (Hidayani et al., 2015; Fujaya et al., 2016 a, b; La Sara., 2017). Indicators include a decline in both the size of crabs caught and the catch volume. To safeguard this crab population, the Indonesian government has issued regulations setting a minimum allowable capture size.

In Cockburn Sound, Western Australia, the reported decline in crab catches since 2000 resulted in closure of the crab fishery in December 2006 (Johnston et al., 2011). Kunsook et al (2014) also reported an exploitation rate of 0.71, higher than the optimal value of 0.33 at Kung Krabaen Bay, in the eastern Gulf of Thailand. They also reported a decrease in the size of mature females from 8.10±0.39 cm to 7.52±1.14 cm. Based on these results, Kunsook et al (2014) proposed a sustainable management program for *P. pelagicus*.

Efforts to domesticate this crab have been initiated by Trijuno et al. (2015) and Fujaya et al (2016b), in order to help maintain the blue swimming crab as a sustainable seafood resource. They have succeeded in breeding *P. pelagicus* seed in a hatchery and raising them in ponds. Growth rates are quite good and the meat of the pond-reared crabs can meet export quality standards.

This study aimed to evaluate the changes resulting from domestication followed by selective breeding for rapid growth in terms of population genetics and growth rate. The

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genetic distance between domesticated crab generations (1st and 4th generation) and changes in growth rate between 1st and 4th generation crabs were analysed. Ways to use the research data to manage the blue swimming crab fishery more sustainably were also considered.

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2. Materials and Methods

This study was conducted from October 2015 to March 2016. Blue swimming crab seed (larvae) were produced at the Hasanuddin University educational hatchery, and grown-out in brackish-water ponds belonging to the South Sulawesi Province Research Development Agency, located in Barru Regency. RAPD analysis was carried out at the Science Building, Faculty of Mathematics and Science, Hasanuddin University, Makassar, South Sulawesi, Indonesia.

Broodstock Collection. Wild crab broodstock were collected from Makassar Strait crab fishermen, and domesticated crab broodstock (3rd generation undergoing selection for fast growth) were collected from the Hasanuddin University experimental pond in Bojo Village, Barru Regency, South Sulawesi Province, Indonesia. Male and female crabs were transported to the Hasanuddin University hatchery using plastic buckets equipped with aerators. The candidate broodstock were then held and prepared for spawning in a 2-ton volume concrete tank equipped with a sand substrate. The tanks were aerated until the crabs spawned. Broodstock were given a commercial feed formulation containing marine worms, sardines, and squid. Feed was provided *ad libitum* twice a day.

Hatching and Larval Rearing. Hatching took place in a concrete tank (1000 L volume). After the eggs hatched, the broodstock were removed and transferred back to the broodstock pond. The larvae were transferred to a larval rearing tank. During rearing, larvae were fed rotifers, artemia nauplii, and artificial feed. The rearing tank water was maintained at a temperature of 30-32 °C, with salinity 31-33 ppt, and dissolved oxygen 4.5 ppm. Shelter in the form of plastic tape was placed in the rearing tank when the larvae entered the megalopa stage. Seed were harvested when the larvae reach the Crab-5 stage (after 5 days) with a carapace width of around 5 mm.

Grow out. Crab seed (crablets) were grown out in brackish-water ponds for 100 days, with a stocking rate of 1/m². Seaweed was scattered into the ponds before the crab seed were stocked at a rate of 1 kg/m². Seaweed was used as a shelter by the crablets. During grow out, the crablets were fed with fish at 2% dry weight per day. Feeding took place twice a day (every morning and evening).

Selective breeding. The selection method used was an individual/mass method following the instructions of Tave (1995). Prospective brood stock were collected from several populations to form a brood stock base population in order to obtain larvae (crablets). The crablets from different individuals within the population were pooled (mixed together) and grown out in ponds for 3 months before undertaking the selection of individuals with above-average growth. The selected (fastest growing) individuals were then maintained as prospective broodstock for the next generation. Performance, especially growth, was analysed as an indicator of the increase in genetic potential due to this selection process. This increase can also be called the "genetic gain" of the crabs produced (Figure 1).

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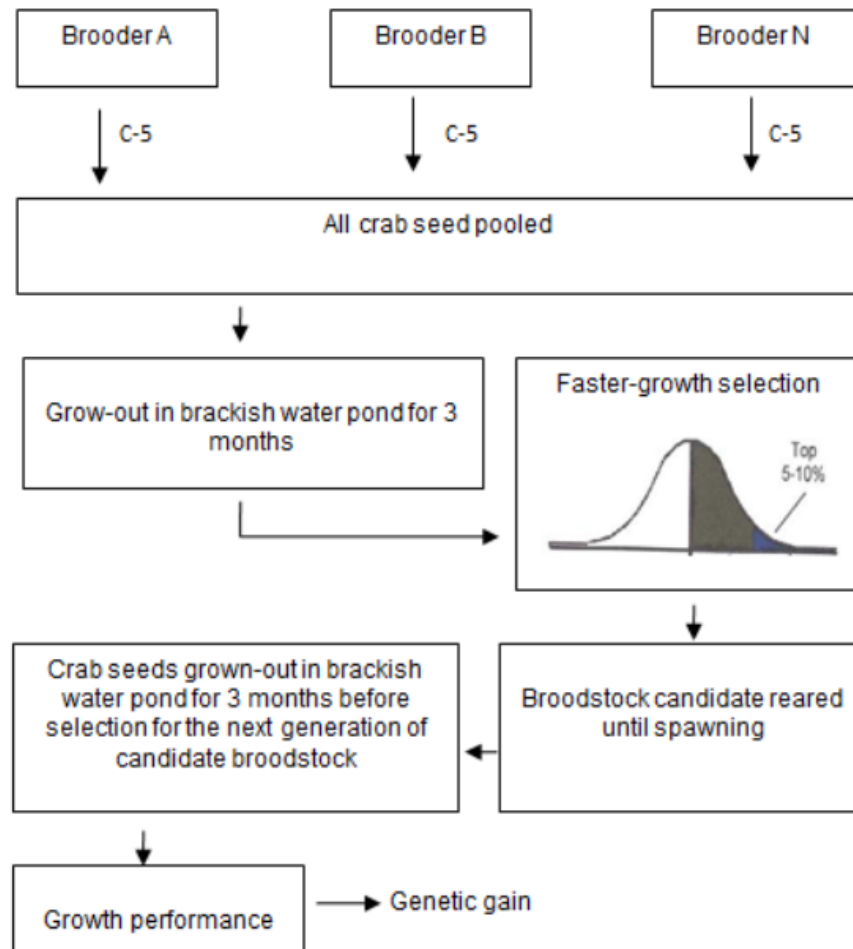


Figure 1. Flow Diagram of selection for fast growth (After Fujaya et al., 2016b).

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RAPD Analysis. The Random Amplification of Polymorphic DNA (RAPD) analysis was carried out on the 1st and 4th generations of domesticated crabs selected for fast growth. The process comprised 4 main stages: 1) collection of tissue samples; 2) genomic DNA extraction; 3) RAPD-PCR Assay; and 4) analysis of the DNA bands formed.

Tissue samples (claw muscle tissue) for the RAPD analysis were collected from the claws of surviving male crabs after grow-out in ponds for 100 days. The blue swimming crab samples could be divided into 4 groups, namely: G1B (1st generation, big size), G1S (1st generation, small size), G4B (4th generation, big size), and G4S (4th generation, small size). The crabs were anaesthetised with ice water at 8°C before claw tissue (meat) removal. Each sample was placed in a labelled bottle filled with 70% ethanol.

Genomic DNA extraction followed the Geneaid Tissue DNA Extraction method. The process comprised the following steps: firstly, 30 mg of tissue sample was placed in an Eppendorf tube, then 200 µL GT buffer and 20 µL Proteinase K were added. The tissue sample was crushed using a Microprestel and then incubated at 60°C for 30 minutes. After adding 200 µl GBT buffer, the tube was then vortexed for 5 seconds and re-incubated at 60°C for 20 minutes until all lysates were destroyed. After incubation, 200 µl of pure ethanol was added followed by vortexing for 10 seconds and transfer to a genomic DNA (GD) column and centrifuging for 2 minutes at 16,000 G. Each sample

was then washed three times. During each wash, the supernatant was transferred to a collecting tube and placed in a GD column to which wash buffer was added (first wash 400 μ L, second and third was 600 μ L) followed by centrifuging at 16,000 G for 30 seconds. After the third wash, the GD column was transferred to a new Eppendorf tube, to which 100 μ L of pre-heated elution buffer was added and allowed to stand for 5 minutes before centrifuging at 16,000 G for 30 seconds. The eluted DNA in the Eppendorf tube was then ready for PCR.

Five universal primers were used for the RAPD-PCR assay. These were the five primers in the Operon Technology Kit A (OPA) (Table 1). A M100bp DNA ladder was also used to calibrate the results.

Table 1. Primers and primer sequences used for the detection of polymorphism in domesticated blue swimming crabs

No	Primer	Sequence	GC content (%)
1	OPA-2	AGG GGT CTT G	60,0
3	OPA-5	TCT GTG CTGG	60,0
3	OPA-11	CAA TCG CCG T	60,0
4	OPA-14	TCT GTG CTGG	60,0
5	OPA-17	GAC CGCTTG T	60,0

DNA amplification reactions were carried out after DNA cleaning using PuReTaq RTG-PCR beads (GE healthcare). PCR was carried out on a PCR GeneAmp System 2700 (Applied Biosystems) unit programmed for 45 cycles. Each PCR reaction tube contained a total volume of 25 μ L, comprised of 1 μ L primer (50 pmol/ μ L), 3 μ L DNA template, and 21 μ L water free RNase mix. The PCR thermocycler was programmed for 45 cycles of 94 °C for 30 sec, 36 °C for 30 sec, and 72 °C for 1 min. An initial denaturation step of 2 min at 94 °C and a final extension step of 2 min at 72 °C were included in the first and last cycles, respectively.

The PCR products were separated through electrophoresis on 2.0% agarose gel in a 1x TBE buffer solution for 1.5 hours at 150 volts. Agarose gel (0.8%) was made through heating a solution of 0.24 g agarose in 30 ml of 1x TBE buffer until clear, then pouring the liquid gel into mould with a comb to form wells for loading PCR product. A 3 μ L volume of migrated PCR product was taken and mixed with 1.0 μ L loading dye, and inserted into each electrophoresis well. Electrophoresis in 1x TBE buffer at 150 Volt with a current strength of 75 mA was run until the DNA had migrated three quarters of the length of the gel from the negative pole towards the positive pole. The gel was then stained with ethidium bromide at a concentration of 0.5 ng/mL. DNA electrophoresis results were visualized under ultra-violet (UV) light and documented by a digital documentation system connected to a computer using a proprietary program.

3. Statistical Analysis

Growth performance was measured as an indicator of the potential for genetic improvement of this crab. Parameters measured were carapace width (CW) and growth rate. The CW was measured as the distance between the tips of the anterolateral carapace spines using digital callipers. Growth rate (mm day⁻¹) was calculated as the difference between final (at time t) and initial CW measurements divided by the rearing time: (CW_t – CW₀) / t⁻¹. Growth rate data were compared using Student's T-test.

Scoring of RAPD-PCR assay fragments was carried out based on predetermined standard markers to obtain binary data. The presence of fragments was scored as 1 (one) while the absence of fragments was scored as 0 (zero). The UPGMA (Unweighted Pair Group Method of Arithmetic) Dendrogram of the *Portunus pelagicus* sample groups was constructed based on Nei (1972) from similarity index and genetic distance values, using the computer program Tools For Population Genetic Analyses (TFPGA Ver. 1.3) (Miller, 1997).

4. Results and Discussion

4.1 Results

The sex ratio (Male:Female) was similar for the first generation (44:56) and fourth generation (45:55). The data on domesticated crab carapace width from the 1st and 4th generations show a shift towards the right (larger sizes) in the size distribution for both sexes (Figure 2). These results indicate that there is a change in mean growth rate between the 1st and 4th generation. The mean growth rate of the 1st generation was 1.0. mm per day while the fourth generation has a faster mean growth rate of 1.1 mm per day (Table 2).

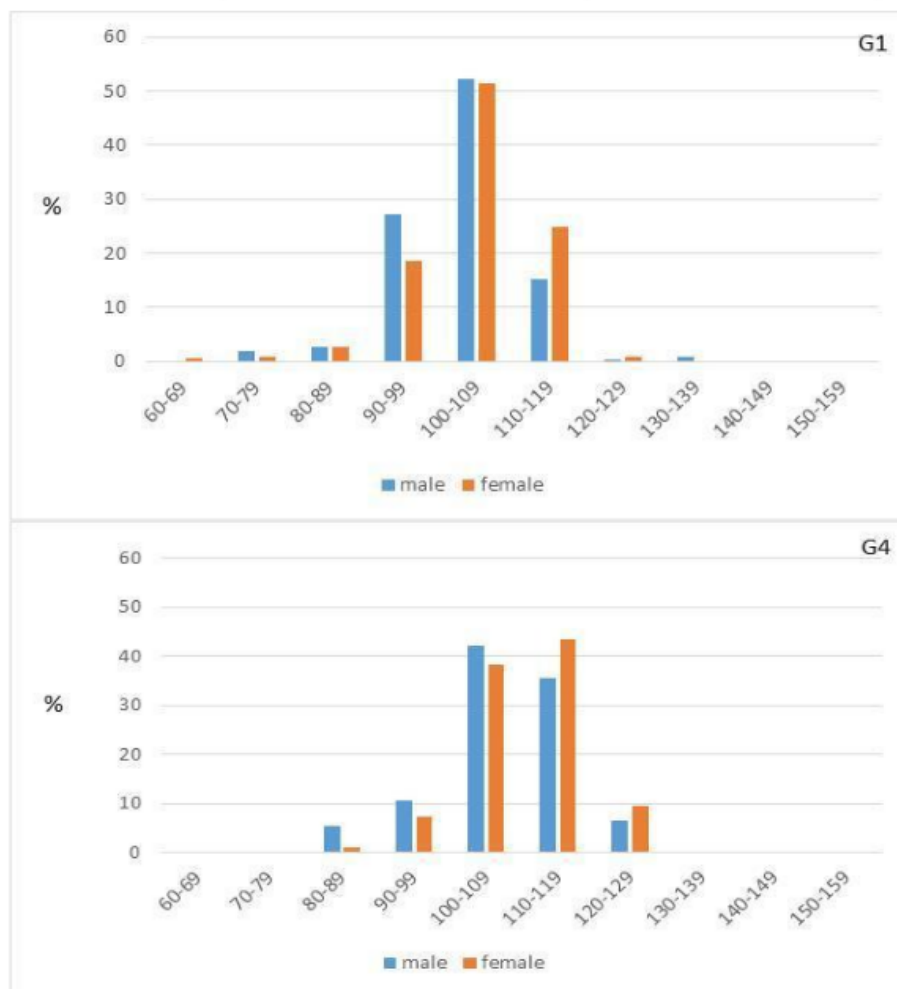


Figure 2. Carapace Width (mm) distribution of 1st (G1) and 4th generation (G4) domesticated crabs

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Table 2. Growth rate of domesticated crabs after 100 days rearing in ponds

Generation	Sex	Growth in Carapace Width (mm)	Growth rate (mm day ⁻¹)
G1	Male	102.0	1.0
	Female	104.1	1.0
G4	Male	107.8	1.1
	Female	109.8	1.1

Harvested crab yields were divided into 4 groups: G1B (1st generation, big size; mean CW = 105 mm), G1S (1st generation, small size; mean CW = 90 mm), G4B (4th generation, big size; mean CW = 115 mm), and G4S (4th generation, small size; mean CW = 100 mm). These four groups were compared using the DNA assay.

The DNA amplification products showed that the universal primer set (OPA) performed well. The OPA-2 primer produced 6-13 fragments DNA with a size of 200-1500 bp; the OPA-5 primer produced 6-8 fragments of 300-900 bp; the OPA-11 primer produced 7-13 fragments of 250-1500 bp; the OPA-14 primer produced 3 fragments of 500-1500 bp; and the OPA-17 primer produced 4-8 fragments of 400-1550 bp. The electrophoresis results from PCR product obtained from *P. pelagicus* DNA using four of the five primers are shown in Figures 3, 4, 5, and 6.

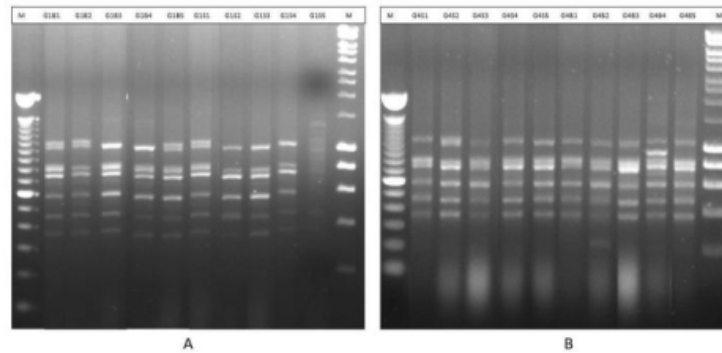


Figure 3. OPA-2 Amplified products on 0.8 % agarose gel. Each lane shows an amplified DNA sample from a different individual from 1st generation (A) and 4th generation (B) crabs. Marker: 1 kb molecular weight

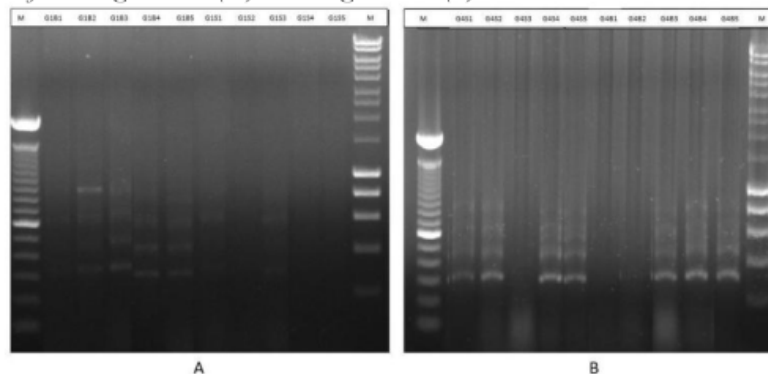


Figure 4. OPA-5 Amplified products on 0.8% agarose gel. Each lane shows an amplified DNA sample from a different individual from 1st generation (A) and 4th generation (B) crabs.. Marker: 1 kb molecular weight

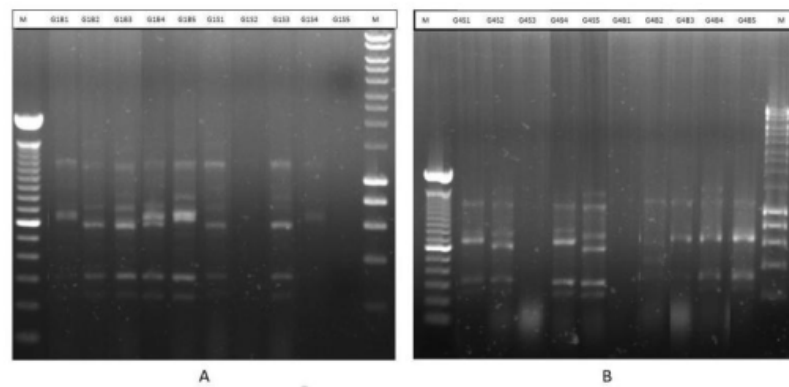


Figure 5. OPA-11 Amplified products on 0.8% agarose gel. Each lane shows an amplified DNA sample from a different individual from 1st generation (A) and 4th generation (B) crabs. Marker: 1 kb molecular weight

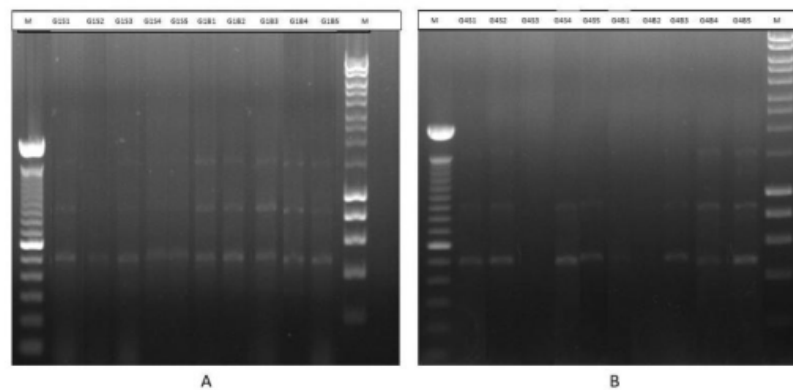


Figure 6. OPA-14 Amplified products on 0.8% agarose gel. Each lane shows an amplified DNA sample from a different individual from 1st generation (A) and 4th generation (B) crabs. Marker: 1 kb molecular weight

The number of fragments, polymorphic proportions, and fragment size from the five primers combined showed that the G1B (big size from 1st generation) group had 26 fragments with polymorphic proportions of 34.61%; the G1S (small size from 1st generation) group had 21 fragments, 28.57% polymorphic; the G4B (big size from 4th generation) group had 22 fragments, 40.9% polymorphic; and the G4S (small size from 4th generation) group had 25 fragments, 36% polymorphic. Overall, the four groups produced fragments varying in size from 250-1550 bp. These data were used to analyse the similarity index and genetic distance.

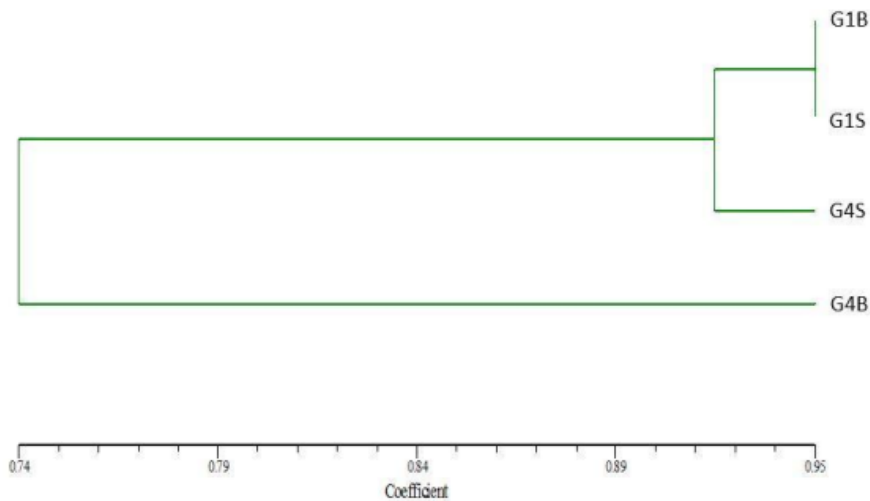
The similarity index and the genetic distances between the four groups (G1B, G1S, G4B, and G4S) indicate significant differences between the G4B group and the other three groups (Tables 3 and 4). The dendrogram using RAPD markers showed two clusters (Figure 7).

Table 3. Similarity Index of *P. pelagicus* groups

Pop. ID	G1B	G1S	G4B	G4S
G1B	1.00			
G1S	0.95	1.00		
G4B	0.74	0.68	1.00	
G4S	0.95	0.89	0.79	1.00

Table 4. Genetic Distance between *P. pelagicus* groups

Pop. ID	G1B	G1S	G4B	G4S
G1B	0.000			
G1S	0.0527	0.000		
G4B	0.2632	0.3158	0.000	
G4S	0.0527	0.1053	0.2106	0.000



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Figure 7. Dendrogram based on Nei's (1972) Genetic distance; UPGMA Method; G1 (1st generation); G4 (4th generation); B big sample; S small sample

4.2 Discussion

Doyle (1983) argues that domestication selection is defined as natural selection in traits that affect survival and reproduction in a human (domestic) controlled environment. Growth rates, disease resistance, tolerance of a given environment, and ability to utilize feed are among the most common targets of selection (Liao and Huang (2000). Wellenreuther et al (2019) found pronounced growth and gene expression differences between wild and domesticated individuals related to global developmental and immune pathways. Temperature-modulated growth responses were linked to major pathways affecting metabolism, cell regulation and signalling. In this study, domestication with selection for rapid growth carried out on the blue swimming crab succeeded in increasing growth performance and resulted in changed genetic distance.

Each animal has a different response to domestication selection. Gjedrem (2016) reports various genetic benefits obtained from large aquaculture breeding programs. For example, in Nile tilapia (*Oreochromis niloticus*) growth rate was doubled after 6-7 generations of selection. Growth rates of Atlantic salmon (*Salmo salar*) in Norway more than doubled after 5 generations of selection for rapid growth. The response was also positively correlated for protein and energy retention so that the FCR was reduced by 23%. Genetic benefits are also reported for several diseases such as resistance to pancreatic necrosis (IPN) and *Vibrio salmonicida* infection. However, Solberg et al., (2013) suggested that domestication could lead to reduced or increased genetic variation in salmon, with a decline in genetic variation increasing susceptibility to diseases and predators in salmon

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12 farms. Trut et al. (2009) describe changes in behaviour, morphology and physiology that appeared during selection for tameness, similar to those observed in the domestic dog. Continued evaluation and interpretation of genetic variation during commercial cultivation on the same species in different environments is important, so that the goals of domestication and the main targets of breeding programs can be achieved. Domesticated populations are susceptible to inbreeding, which is likely to result in an increase homozygote, with potential negative impacts on fitness and traits important for aquaculture. Therefore, it is not always recommended to continue to conduct domestication selection for economically important traits. Cross breeding can be important to re-increase heterozygote and genetic variation in cultivated populations of a species. The results of the Goyard et al (2008) study give good examples of improvements in performance with effects and/or heterosis resulting from cross breeding, as well as loss of performance from inbreeding. They show that the aquaculture industry can benefit from the use of two different strains to produce F1 hybrids in commercial cultivation.

An increase in growth performance was observed, although it was not as large or significant as that obtained through selective breeding of salmon and tilapia (Gjedrem et al., 2016). The data from this study indicate that the blue swimming crab shows good prospects for development as an aquaculture commodity to supply the increasing world market demand. Trijuno et al. (2015) also reported that domesticated blue swimming crab broodstock could produce superior offspring with higher survival rate, faster larval growth changes and more uniform larval growth compared to larvae from wild brood stock. This study showed that the use of domesticated blue swimming crab broodstock can also improve crablet and juvenile crab grow-out performance.

Four possible scenarios are suggested for the future of sustainable blue swimming crab fisheries, making use of blue swimming crab breeding and culture technology. These are as follows: 1) to develop a blue swimming crab aquaculture industry that focuses on brood stock domestication, similar to the path taken by agriculture but avoiding the negative impacts of captive breeding by continuing to evaluate and control genetics; 2) to restock wild population with blue swimming crab seed obtained from natural (wild-caught) brood stock; 3) scientists and aquaculture organizations should inform farmers about the great benefit of using genetically improved stocks; and 4) government should stimulate as well as financially support the establishment of breeding programs and restocking.

5. Conclusion

Domestication followed by selection for fast growth resulted in a change in genomic DNA characters associated with the positive trait of faster growth. Domestication with selection for fast growth can be used to produce domesticated blue swimming crab broodstock which will produce offspring with higher growth rates. Domestication with selection for fast growth as carried out under this study can still be continued past the 4th generation (G4), because genetic variation in the G4 population is still quite high.

23 6. Acknowledgements

9 The authors wish to thank the Ministry of Education and Culture, Republic of

Indonesia, for funding this research through a grant under the Excellent National Strategic Research scheme. In-kind support provided by the Faculty of Marine Sciences and Fisheries, Hasanuddin University and by the Research Development Agency, South Sulawesi Province, is gratefully acknowledged.

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